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Display  ☒ Show  ☒ Send to ☒ Hide: ☐ sequence ☐ all but gene, CDS and mRNA

Range: from  to  ☐ Reverse complemented strand Features: ☒

☐ 1: [X04164](#). Reports *Bacillus subtilis*...[gi:40116]

[Links](#)

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LOCUS X04164 353 bp DNA linear BCT 04-SEP-1991  
 DEFINITION *Bacillus subtilis* rrnO operon promoter and leader region.  
 ACCESSION X04164  
 VERSION X04164.1 GI:40116  
 KEYWORDS 16S ribosomal RNA; inverted repeat; ribosomal RNA; rrnO operon.  
 SOURCE *Bacillus subtilis*  
 ORGANISM *Bacillus subtilis*  
 Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.  
 REFERENCE 1 (bases 1 to 353)  
 AUTHORS Ogasawara,N., Moriya,S. and Yoshikawa,H.  
 TITLE Structure and organization of rRNA operons in the region of the replication origin of the *Bacillus subtilis* chromosome  
 JOURNAL Nucleic Acids Res. 11 (18), 6301-6318 (1983)  
 PUBMED 6312418  
 COMMENT For rrnO operon 3'end see <X04166>.  
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 Location/Qualifiers  
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 /organism="Bacillus subtilis"  
 /mol\_type="genomic DNA"  
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 promoter 150..155  
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ORIGIN

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181 acaagttctt tgaaaactaa acaagacaaa acgtacctgt taattcattt ttataaatcg
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301 gccgcaggat gcggtgaact taacctgtga tccatttatc ggagagtttg atc
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Jun 19 2007 13:56:00

LOCUS X04164 6 bp DNA linear BCT 04-SEP-1991  
DEFINITION Bacillus subtilis rrnO operon promoter and leader region.  
ACCESSION X04164 REGION: 30..35  
VERSION X04164.1 GI:40116  
KEYWORDS 16S ribosomal RNA; inverted repeat; ribosomal RNA; rrnO operon.  
SOURCE Bacillus subtilis  
ORGANISM Bacillus subtilis  
Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.  
REFERENCE 1 (bases 1 to 6)  
AUTHORS Ogasawara,N., Moriya,S. and Yoshikawa,H.  
TITLE Structure and organization of rRNA operons in the region of the  
replication origin of the Bacillus subtilis chromosome  
JOURNAL Nucleic Acids Res. 11 (18), 6301-6318 (1983)  
PUBMED 6312418  
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☐ 1: [X04164](#). Reports *Bacillus subtilis*...[gi:40116]

[Links](#)

[Comment](#)   [Features](#)   [Sequence](#)

LOCUS            X04164                            6 bp       DNA       linear       BCT 04-SEP-1991  
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ACCESSION    [X04164](#) REGION: 52..57  
VERSION      X04164.1    GI:40116  
KEYWORDS     16S ribosomal RNA; inverted repeat; ribosomal RNA; rrnO operon.  
SOURCE       *Bacillus subtilis*  
      ORGANISM   [Bacillus subtilis](#)  
                 Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.  
REFERENCE    1    (bases 1 to 6)  
      AUTHORS    Ogasawara,N., Moriya,S. and Yoshikawa,H.  
      TITLE      Structure and organization of rRNA operons in the region of the  
                 replication origin of the *Bacillus subtilis* chromosome  
      JOURNAL    Nucleic Acids Res. 11 (18), 6301-6318 (1983)  
      PUBMED     6312418  
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[X04164](#). Reports *Bacillus subtilis*...[gi:40116][Links](#)[Comment](#)   [Features](#)   [Sequence](#)

LOCUS            X04164                            6 bp     DNA       linear     BCT 04-SEP-1991  
DEFINITION     *Bacillus subtilis* rrnO operon promoter and leader region.  
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VERSION       X04164.1    GI:40116  
KEYWORDS       16S ribosomal RNA; inverted repeat; ribosomal RNA; rrnO operon.  
SOURCE         *Bacillus subtilis*  
         ORGANISM   [Bacillus subtilis](#)  
                     Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.  
REFERENCE       1    (bases 1 to 6)  
         AUTHORS   Ogasawara,N., Moriya,S. and Yoshikawa,H.  
         TITLE       Structure and organization of rRNA operons in the region of the  
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         JOURNAL     Nucleic Acids Res. 11 (18), 6301-6318 (1983)  
         PUBMED       [6312418](#)  
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ORIGIN  
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☐ 1: [X04164](#). Reports *Bacillus subtilis*...[gi:40116]

[Links](#)

Comment   Features   Sequence

LOCUS            X04164                            6 bp       DNA       linear       BCT 04-SEP-1991  
DEFINITION    *Bacillus subtilis* rrnO operon promoter and leader region.  
ACCESSION    [X04164](#) REGION: 150..155  
VERSION      [X04164.1](#)    GI:40116  
KEYWORDS     16S ribosomal RNA; inverted repeat; ribosomal RNA; rrnO operon.  
SOURCE       *Bacillus subtilis*  
      ORGANISM    [Bacillus subtilis](#)  
                  Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.  
REFERENCE    1 (bases 1 to 6)  
      AUTHORS    Ogasawara,N., Moriya,S. and Yoshikawa,H.  
      TITLE      Structure and organization of rRNA operons in the region of the  
                  replication origin of the *Bacillus subtilis* chromosome  
      JOURNAL    Nucleic Acids Res. 11 (18), 6301-6318 (1983)  
      PUBMED     [6312418](#)  
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## Links

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LOCUS       V01490                28 bp    DNA         linear    BCT 04-SEP-1991
DEFINITION  B. subtilis replication origin region and gene rrnO start.
ACCESSION   V01490 REGION: 102..129
VERSION     V01490.1  GI:40114
KEYWORDS    16S ribosomal RNA; origin of replication; ribosomal RNA.
SOURCE      Bacillus subtilis
            ORGANISM  Bacillus subtilis
                        Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 28)
AUTHORS     Ogasawara,N., Seiki,M. and Yoshikawa,H.
TITLE       Replication origin region of Bacillus subtilis chromosome contains
            two rRNA operons
JOURNAL     J. Bacteriol. 154 (1), 50-57 (1983)
PUBMED      6187731
COMMENT     Data kindly reviewed (12-SEP-1983) by H. Yoshikawa.
FEATURES             Location/Qualifiers
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□ 1: [V01490](#). Reports *B. subtilis* repli...[gi:40114]

[Links](#)

Comment   Features   Sequence

LOCUS            V01490                            29 bp       DNA       linear       BCT 04-SEP-1991  
DEFINITION      B. subtilis replication origin region and gene rrnO start.  
ACCESSION       [V01490](#) REGION: 199..227  
VERSION          V01490.1    GI:40114  
KEYWORDS        16S ribosomal RNA; origin of replication; ribosomal RNA.  
SOURCE           Bacillus subtilis  
    ORGANISM     [Bacillus subtilis](#)  
                  Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.  
REFERENCE       1    (bases 1 to 29)  
    AUTHORS      Ogasawara,N., Seiki,M. and Yoshikawa,H.  
    TITLE        Replication origin region of Bacillus subtilis chromosome contains  
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    JOURNAL      J. Bacteriol. 154 (1), 50-57 (1983)  
    PUBMED       [6187731](#)  
COMMENT          Data kindly reviewed (12-SEP-1983) by H. Yoshikawa.  
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Items 1 - 6 of 6

One page.

- ☐ **1:** [D26185](#) Reports Links  
Bacillus subtilis gene, 180 kilobase region of replication origin  
gi|467326|dbj|D26185.1|BAC180K[467326]
- ☐ **2:** [AY618310](#) Reports Links  
Integration vector pDG3661, complete sequence  
gi|49036565|gb|AY618310.1|[49036565]
- ☐ **3:** [X04164](#) Reports Links  
Bacillus subtilis rrnO operon promoter and leader region  
gi|40116|emb|X04164.1|[40116]
- ☐ **4:** [X04166](#) Reports Links  
Bacillus subtilis 5S rRNA gene (3' term. of rrnO operon)  
gi|40115|emb|X04166.1|[40115]
- ☐ **5:** [V01490](#) Reports Links  
B. subtilis replication origin region and gene rrnO start  
gi|40114|emb|V01490.1|[40114]
- ☐ **6:** [V01489](#) Reports Links  
B. subtilis 5' end of rDNA from the 3.1Kb EcoRI fragment including 2.6Kb PstI, 1.9Kb HindIII fragments. This rrn gene set has not been mapped and has no letter designation (as do rrnA, rrnB, and rrnO of B. subtilis)  
gi|40092|emb|V01489.1|[40092]

Items 1 - 6 of 6

One page.

Display Summary 

# Regulated Operon: *rrnO-16S-trnO-Ile-trnO-Ala-rrnO-23S-rrnO-5S*

## Genes

Genes	Synonyms	Direction	Genome position	Function	COG ID
rrnO-16S		+	9809..11361	ribosomal RNA-16S	
trnO-Ile		+	11462..11538	transfer RNA-Ile	
trnO-Ala		+	11550..11625	transfer RNA-Ala	
rrnO-23S		+	11707..14634	ribosomal RNA-23S	
rrnO-5S		+	14690..14801	ribosomal RNA-5S	

*Search notes*

**Operon evidence:** agrees with ribosomal RNA 5' sequence

**Reference:** Ogasawara N, et al. (1983). Sogin ML & Pace NR (1976)

**Comments:**

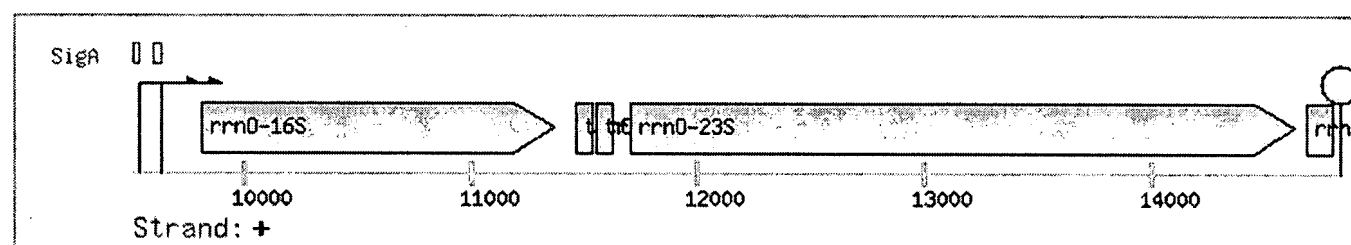
## Promoters

Binding factor	Regulation	Location	Absolute position	Binding seq.(cis-element)
SigA	Promoter	-46:+17	9493..9555	TGTCATAACCCCTTTACAGTCATAAAAAATTATGGTATAATCATTTCTGTTGTCTTTTAA
SigA	Promoter	-45:+18	9591..9653	CAAAAAAGTATTGACCTAGTTAACTAAAAATGTTACTATTAAGTAGTCGCTTTGAGAG

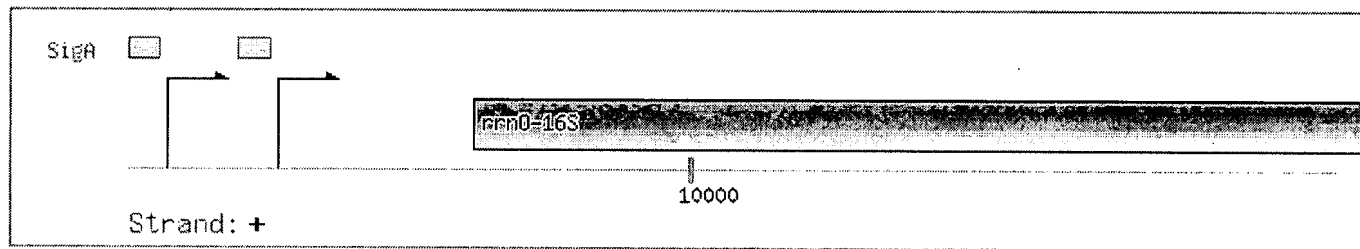
## Terminator

Terminator sequence	Absolute position	Position from stop codon	Free energy [kcal/mol]	Downstream of
TTAAACCCAGCTCAATGAGCTGGGTTTTTGTGTTGTTAA >>>>>>> <<<<<<<<	14815..14833	14..32	-15.7	rrnO-5S

## Overview



## Upper Region

**DBTBS**

Copyright: Human Genome Center, Inst. Med. Sci., Univ. Tokyo; 1999-20

Contact: [Kenta Nakai](#)

## WEST Search History

DATE: Wednesday, August 08, 2007

Hide? Set  
       Name Query

*DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=OR*

- ☐ L22 112.clm. same (\$paraspor\$ or endospor\$ or spore\$ or \$spore).clm.
- ☐ L21 120 and (\$paraspor\$ or endospor\$ or spore\$ or \$spore).clm.
- ☐ L20 L19 and (strong near5 promoter)
- ☐ L19 L17 and promoter
- ☐ L18 L17 and l3
- ☐ L17 L16 and l13
- ☐ L16 L12 same (domain\$ or moiet\$ or fragment\$ or region\$ or cell-binding\$ or receptor-binding\$)
- ☐ L15 L14 and (domain or moiety or fragment or region or cell-binding or receptor-binding)
- ☐ L14 L13 and l12
- ☐ L13 (bacil\$ or subtil\$) same (\$paraspor\$ or endospor\$ or spore\$ or \$spore)
- ☐ L12 clostrid\$ or tetan\$
- ☐ L11 L10 and \$spore
- ☐ L10 L9 and \$toxin
- ☐ L9 fragment.clm. near c.clm.
- ☐ L8 l6 and l4
- ☐ L7 L6 and l5
- ☐ L6 l3 same promoter
- ☐ L5 l4 and (bacil\$ or subtil\$)
- ☐ L4 l3 and (\$paraspor\$ or endospor\$ or spore\$)
- ☐ L3 ttaca
- ☐ L2 AGAAGAACAAGAAGAAGTGTGAAAAAAGCGCAGCTGAAATAGCTGCGCTTT
- ☐ L1 AGAAGAACAAGAAGAAGTGTGAAAAAAGCGCAGCTGAAATAGCTGCGCTTT  
     AAAATTATGGTATAATCATTCTgTTGTCTTTTAAAGAC

END OF SEARCH HISTORY

## WEST Search History

DATE: Wednesday, August 08, 2007

Hide?	<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>
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<input type="checkbox"/>	L12	L11 and (\$spor\$)	39
<input type="checkbox"/>	L11	L10 and (fusion or fused or link\$ or coupl\$ or covalent\$ or join\$).ti,ab,clm.	282
<input type="checkbox"/>	L10	L9 and (receptor or binding or c-fragment or fragment-c or (fragment near2 c))	412
<input type="checkbox"/>	L9	L8 and fragment\$.ti,ab,clm.	518
<input type="checkbox"/>	L8	tetan\$.ti,ab,clm.	2244
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END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 20050142151 A1

TITLE: Immunogenic compositions including rough phenotype Brucella host strains and complementation DNA fragments

Summary of Invention Paragraph:

[0019] Preferably, the heterologous antigen is selected from the group consisting of: Anthrax antigens such as Bacillus anthracis protective antigen (PA), inactive variants of Edema Factor and Lethal Factor; plague antigens such as Yersinia pestis F1 and V antigens and F1-V fusion proteins; malaria proteins such as circumsporozoite and merozoite antigens, and including antigens of Plasmodium berghei (sporozoite and merozoite antigens), Plasmodium falciparum, Plasmodium vivax and Plasmodium malariae, including CSP and MSP1 antigens of all of these; Francisella antigens, particularly from Francisella tularensis; staphylococcal and streptococcal enterotoxin fragment antigens; Burkholderia antigens; Coxiella antigens; Clostridium epsilon toxoids; botulinum toxoids; smallpox antigens; mycobacterial antigens; cancer antigens; HIV antigens; tetanus toxoids (including TetC); diphtheria toxoids; pertussis toxoid; Helicobacter antigens; Borrelia antigens; Legionella antigens; Bartonella antigens; vaccinia antigens; antigen-GFP fusions; tagged antigens (6his, V5, etc.), fusions of antigens to secretory signals, fusions of antigens to each other; genes encoding therapeutic molecules or enzymes producing therapeutic molecules; antigens from other parasites, antigens from viruses, and the like. Also contemplated are heterologous genes encoding enzymes that would not serve directly as antigens but would instead synthesize non-protein products in the Brucella platform that would themselves function as heterologous antigens--for instance, lipids and polysaccharides. In addition, homologous antigens can be included to enhance immunogenicity.

Detail Description Paragraph:

[0055] Brucella, even attenuated strains, penetrate to the liver. Defense against malaria may involve destruction of liver stage parasites to prevent infection or attack on merozoites to reduce disease. Presence of antibody and Th-type 1 cellular responses, characterized by CD4 and CD8 T-lymphocytes directed at circumsporozoite protein (CSP), are associated with prevention of patent infection after challenge with P. berghei, an agent of murine malaria, and P. falciparum, which causes a severe form of human malaria. Immune response to merozoite surface protein-1 (MSP-1) of these parasites is associated with reduction in patent infection intensity. Since P. falciparum does not cause malaria in mice, the murine P. berghei model is used to demonstrate initial proof of concept for malaria vaccine approaches. Vaccines that work in the P. berghei model can be reconstructed with P. falciparum antigens. Safety and immunogenicity testing in mice and efficacy testing against blood stage infection in nonhuman primates can then lead to human trials. Department of Immunology efforts are directed toward enhancing the potency of immune response against CSP, MSP-1 and other malarial antigens. Genes for these antigens were cloned into expression vectors for use as DNA vaccines and for production of recombinant proteins for clinical vaccine tests and in vitro studies. Movement of these genes into pBBR1MCS should be readily accomplished.

Detail Description Paragraph:

[0068] As a prototype, we constructed a live, attenuated B. melitensis vaccine strain that expresses protective antigens from three known threat agents and tested it for safety, immunogenicity and protective efficacy in appropriate animal models. For instance, genes encoding some or all of the following can be used: a) protective antigen (PA) from Bacillus anthracis, b) protective C fragment of tetanus toxin and c) protective fragments of V antigen from Yersinia pestis. These antigens may be encoded by a plasmid that also encodes a Brucella gene (wboA), which, as noted above, is preferred for assisting survival of the vaccine strain in vivo and to some extent, protective immunity.

Detail Description Paragraph:

[0070] Malarial antigens are also contemplated. A Mycobacterium bovis antigen expressed on broad host range plasmid pBBR1MCS in Brucella abortus strain RB51 produced serum antibody in mice and antigen-stimulated IFN-gamma by splenocytes [Vemulapalli, Infect Immun. 68:3290-6, 2000]. Based on our own data, expressing Plasmodium antigens from plasmids in our candidate strains will likely induce strong immune responses. Defense against malaria may involve destruction of liver stage parasites to prevent infection or attack on merozoites to reduce disease. Presence of antibody and Th-type 1 cellular responses directed at circumsporozoite protein (CSP) are associated with prevention of patient infection after challenge with P. berghei, an agent of murine malaria, and P. falciparum, which causes a severe form of human malaria. The murine P. berghei model is used to demonstrate initial proof of concept for malaria vaccine approaches. Immune response to merozoite surface protein-1 (MSP-1) of Plasmodium parasites is associated with reduction in patent infection intensity. With this invention, our efforts are directed toward enhancing the potency of immune response against CSP, MSP-1 and other malarial antigens. Recombinant genes for these antigens were cloned in expression plasmids for use as DNA vaccines and for recombinant protein production in bacteria. Expression of these recombinant genes into Brucella has been accomplished using appropriate Brucella promoters. For instance, purER promoter gives low-level constitutive expression, while Kan promoter gave high constitutive expression, and groES promoter gave intermediate expression (but was inducible late). All three promoters are useful, although Kan is preferred.

#### CLAIMS:

1. An immunogenic composition comprising a live Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a promoter recognizable by Brucella, and (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell
2. The immunogenic composition of claim 1, wherein the Brucella host cell comprises a Brucella DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.
9. The immunogenic composition of claim 1, wherein the complementation DNA fragment comprises the wboA gene.
10. The immunogenic composition of claim 9, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.
11. An immunogenic composition comprising a live attenuated Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a DNA fragment operably linked to a first promoter recognizable by Brucella, and encoding a heterologous antigen; and (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by Brucella, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.
12. The immunogenic composition of claim 11, wherein the Brucella host cell comprises a Brucella DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

19. The immunogenic composition of claim 11, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, *Yersinia pestis* F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, *Plasmodium berghei* antigens, *Plasmodium falsiparum* antigens, *Plasmodium vivax* antigens, *Plasmodium malariae* antigens, *Francisella* antigens, staphylococcal and streptococcal enterotoxin fragment antigens; *Burkholderia* antigens, *Coxiella* antigens, *Clostridium epsilon* toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, *Helicobacter* antigens, *Borrelia* antigens, *Legionella* antigens, *Bartonella* antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, fusions of antigens to secretory signals, and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.
22. The immunogenic composition of claim 19, wherein the DNA fragment of (i) encodes an enzyme synthesizes lipids and/or polysaccharides.
23. The immunogenic composition of claim 11, wherein the complementation DNA fragment comprises the wboA gene.
24. The immunogenic composition of claim 23, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.
25. A vaccine against infection by brucellosis, comprising a live *Brucella* host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated *Brucella*, wherein the host cell is transformed with a recombinant DNA construct replicable in *Brucella*, which DNA construct comprises: (i) a promoter recognizable by *Brucella*, and (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.
26. The vaccine of claim 25, wherein the *Brucella* host cell comprises a *Brucella* DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.
33. The vaccine of claim 25, wherein the complementation DNA fragment comprises the wboA gene.
34. The vaccine of claim 33, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.
36. A vaccine against infection by brucellosis and/or a non-brucellosis disease, comprising a live attenuated *Brucella* host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated *Brucella*, wherein the host cell is transformed with a recombinant DNA construct replicable in *Brucella*, which DNA construct comprises: (i) a DNA fragment operably linked to a first promoter recognizable by *Brucella*, and encoding a heterologous antigen, and (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by *Brucella*, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.
37. The vaccine of claim 36, wherein the *Brucella* host cell comprises a *Brucella* DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.



44. The vaccine of claim 36, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, Yersinia pestis F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, Plasmodium berghei antigens, Plasmodium falsiparum antigens, Plasmodium vivax antigens, Plasmodium malariae antigens, Francisella antigens, staphylococcal and streptococcal enterotoxin fragment antigens; Burkholderia antigens, Coxiella antigens, Clostridium epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, Helicobacter antigens, Borrelia antigens, Legionella antigens, Bartonella antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, fusions of antigens to secretory signals, and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.

47. The vaccine of claim 36, wherein the complementation DNA fragment comprises the wboA gene.

48. The vaccine of claim 47, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

50. A recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a promoter recognizable by Brucella, and (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in a host cell transformed therewith.

51. The recombinant DNA construct of claim 50, wherein the complementation DNA fragment comprises the wboA gene.

52. A recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a DNA fragment operably linked to a first promoter recognizable by Brucella, and encoding a heterologous antigen, and (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by Brucella, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in a host cell transformed therewith.

53. The recombinant DNA construct of claim 52, wherein the complementation DNA fragment comprises the wboA gene.

54. The recombinant DNA construct of claim 52, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, Yersinia pestis F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, Plasmodium berghei antigens, Plasmodium falsiparum antigens, Plasmodium vivax antigens, Plasmodium malariae antigens, Francisella antigens, staphylococcal and streptococcal enterotoxin fragment antigens; Burkholderia antigens, Coxiella antigens, Clostridium epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, Helicobacter antigens, Borrelia antigens, Legionella antigens, Bartonella antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, fusions of antigens to secretory signals, and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.

59. A method for inducing protective immunity to brucellosis in a mammal comprising the step of administering to a mammal a vaccine comprising a live Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a promoter recognizable by Brucella, and (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the

host cell.

60. The method for inducing protective immunity of claim 59, wherein the complementation DNA fragment comprises the wboA gene.

61. The method for inducing protective immunity of claim 60, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

63. A method for inducing protective immunity to brucellosis or a non-brucellosis disease, or both, in a mammal comprising the step of administering to a mammal a vaccine comprising a live Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a DNA fragment operably linked to a first promoter recognizable by Brucella, and encoding a heterologous antigen, and (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by Brucella, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.

64. The method for inducing protective immunity of claim 63, wherein the complementation DNA fragment comprises the wboA gene.

65. The method for inducing protective immunity of claim 4964 wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

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## Education

- B.A., 1957, New York University
- Ph.D., 1961, Columbia University
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## Research Interest

### • **Differential Expression of Ribosomal and Transfer RNA Genes in *Bacillus subtilis* and**

Our research deals with the heterogeneity in organization of the conserved and redundant rRNA (*rrn*) operons and tRNA (*trn*) genes, their expression and the molecular mechanism involved in the regulation of ribosome synthesis in *Bacillus subtilis*. Specifically, we deal with the evolutionary importance for the endospore-forming bacteria for possessing a high clustering of *rrn* operons near the origin of replication and large tRNA gene clusters (2-21) downstream of all 10 ribosomal RNA gene sets. Studies on the differential expression of seven out of the 10 *rrn* operons with single-copy *lacZ* fusions capable of integrating at the native *rrn* site or at heterologous loci (*amyE*, *thrC*) have shown that all are under growth-rate control and are grouped into strong (***rrnO***, *rrnW*), intermediate (*rrnA*, *rrnJ*, *rrnE*), and weak (*rrnD*, *rrnB*) the latter are associated with large cluster of 16 and 21 tRNA gene clusters respectively. We are investigating the role and interaction of the tandemly arranged **promoters** (P1, P2), the Upstream Activating Sequences (UP and UAS) and the 7 bp discriminator sequence in growth-rate regulation and in the stringent control using cloned individual promoter elements without the UAS and after mutating critical regulatory regions of selected *rrn* operons. We follow stable RNA synthesis,  $\beta$ -galactosidase measurements and (p)ppGpp accumulations in cells with genetic backgrounds that are *relA*<sup>+</sup>, *relA*<sup>-</sup>, *relA*(S) and *rpoB* grown as a function of different growth rates, during amino acid starvation or carbon source limitation.

Our second project deals with how bacterial populations specifically various *Bacilli* can behave in an organized manner to generate highly geometrically morphologies or morphotypes on solid and semi-solid surfaces. Five morphotypes have been generated for *B. subtilis* under nutrient scarcity and hardness of the agar surface: 1) the common compact round colonial growth with rough edges, (B); 2) tree branches with tip splitting growth, (T); 3) curled or chiral growth with the same handedness branches, (C); 4) vortex branched growth led by bacteria droplets that spin around a common center, (V); that 5) at times become spiral vortex (SV). These forms are stably inherited exhibiting many physiological and genetic properties distinct from *B. subtilis*. Sequencing of the 16S rDNA gene and Southern hybridization suggests that the colonial patterns may be the result of another *Bacilli* coexisting with *B. subtilis* which is activated during certain hostile conditions. Work is on going to understand the genetic basis of morphotypes and the role played by chemotaxis in generating and maintaining these striking differentiated structures.